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Relative efficiency of hand-sorting, formalin application and combination of both methods in extracting Lumbricidae from Finnish soils[1])

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With 2 figures

1. Introduction

Hand-sorting and formalin application can be regarded as routine sample methods when Lumbricidae populations are surveyed. RAW (1959) pointed out the superiority of formalin application to hand-sorting in recovering individuals of *Lumbricus terrestris*, but for the other species in the samples lower estimates were gained when formalin method was adopted. For studies on quantitative aspects of Lumbricidae populations, formalin application is not considered as an appropriate method since even within the same species its efficiency is not equal in different spells of the year (BOUCHÉ 1969), aestivating individuals cannot be expelled by it and different properties of the soils have their impact upon the efficiency (SATCHELL 1969).

NELSON & SATCHELL (1962) tested the efficiency of hand-sorting by using prepaired soil samples. They suggested that underestimates in the numbers of small earthworm species, especially in those having dark coloration, are likely to occur, but for the adults and the subadults of big and medium sized species hand-sorting yielded adequate estimates.

AXELSSON et al. (1971) re-sorted soil samples to test the efficiency of hand-sorting method. Their results indicate that depending on the type of the soil 13—25 % of the juveniles will be left undiscovered in the samples after the first sorting, but to recover the adults even the first sorting gives adequate result.

ZICSI (1962) concluded that hand-sorting of a sample plot of 1/16 m^2 taken to the depth of 20 cm is enough to gain rather good estimates for medium sized species but for bigger species a larger and deeper sample unit is required.

BOUCHÉ (1969) applied formalin solution to the sample plot prior to digging up the soil material and examining it in the laboratory. This yielded both the higher number of individuals and the total biomass than formalin method alone. Moreover, it indicated lower density of individuals but higher total biomass than by mechanical sorting of the soil without formalin application.

Liquid vermifuges make the unaestivating earthworms wriggle, stretch out and some of them come to the surface of the soil. Accordingly, one can expect that the individuals irritated this way will be more easy to record in the soil material dug up than those not irritated. On the other hand, maybe some of them can avoid being captured by moving out of the sample plot inside the soil.

In Finland the Lumbricidae fauna is poor in species but the high density of individuals in many habitats makes the Lumbricidae an important part of the soil fauna. The present study was aimed at determining in the field the relative efficiency of formalin application, hand-sorting and combination of both methods in extracting Lumbricidae from different types of Finnish soils. The recovery of the species, the numbers of the individuals and the biomass of the species in different habitats were considered to assess the methods.

1) Communication to the Vth International Colloquium on Terrestrial Oligochaeta, Moscow, 6—10 October 1980.

2. Material and methods

In September 1977 the author sampled Lumbricidae in the main island of the Åland archipelago in SW Finland and in September—October 1979 in coastal area lying about 40—60 km west of Helsinki in S Finland. The numbers of the locations sampled were 47 and 34, respectively, but 3 of them were devoid of Lumbricidae.

Two sample plots were dug up in each location. The soil of one sample plot was sorted out by hand on a plastic sheet (HS method), but to the other plot 10 litres of water containing 50 ml of 40 per cent formalin was added (3—4 applications), the earthworms expelled inside the frame limiting the sample plot were collected and preserved in 70 % ethanol (F method) and, after about 5 minutes, the organic soil layer was quickly dug up and handsorted. The data for the combined method (FHS) were received by adding the numbers of the individuals by F to those recovered by handsorting the soil of the same plot.

In each location the two sample plots did not lie further than 1—3 metres from each other and it took 1—2 hours to examine each plot measuring 25 × 50 cm. The organic soil layer was removed down to the mineral soil or e.g. in habitats with dense clay of several metres thick to the depth of about 40 cm. The uppermost part of the soil-with roots of plants were kept separate from other soil material and examined with great accurancy.

The habitats sampled:

1. Alluvial sea shores **(Sh)**, which are open, meadow like habitats without trees or bushes and characterized by very dense, wet and occasionally water-logged mat of grasses and herbs. The mat is very difficult to break into pieces for closer examination. In most locations the organic soil layer was only 5—10 cm thick and there was clay, silt, stones or a mixture of them all lying underneath.

2. Shore alder thickets **(Al)** frame the uppermost parts of alluvial seashores. The organic soil usually reaches to the depth of 10—20 cm, its moisture content is high and it is covered by abundance of grasses, herbs and quickly decaying organic matter. The numerous, often rather thick roots of bushes and trees made it sometimes difficult quickly to dig up the soil material.

3. Meadows **(Me)** are often derived from abandoned fields and have dense vegetation of herbs and grasses but trees and bushes are absent. The organic soil layer is several tens of centimetres thick consisting of either mull or clay mixed with silt. In some locations the heavy clay layer was several metres thick.

4. Rich forests **(RF)** are luxurious, foliaceous forests with great abundance of herbs, grasses, bushes and foliaceous frees. The *Corylus* stands sampled in Åland fall under this category. The organic soil layer is 20—40 cm thick, rich with mull or clay or a mixture of them both and with abundance of decaying organic matter. The *p*H of the soil is high and the roots of the trees made it sometimes difficult to take the sample.

5. Coniferous forests **(CF)** are mostly represented by *Myrtillus* type of forests with podsolic soil profile and acid raw humus. The ground layer is rich with mosses and in the field layer *Vaccinium myrtillus* is the dominate plant species.

More detailed data on the locations sampled in Åland are given in the paper by Haeggström & Terhivuo (1979).

The study material consists of 3.578 individuals belonging to 11 species. The earthworms preserved were identified after 2—3 months, new solution of 70—80 % ethanol was added and the individuals were weighed in groups after 3—4 months from the sampling periods. Prior to weighing the individuals were kept about 2 minutes on blotting paper and in each species the undamaged adults and the juveniles as well as the damaged or the fragments of the adults and the juveniles present in the same sample were weighed up in separate groups. The weight loss due to the preservation of the individuals was not considered. The weights of the fragments and the damaged individuals are included in the total biomass of the species by the methods but special attention was paid not to overestimate the numbers of the individuals the fragments belonged to.

To determine the mean weight of the adults and the juveniles of different species by each method the weights of the fragments and the damaged individuals were subtracted from the corresponding total weights and this was then divided by the number of the undamaged adults or the juveniles.

The study material is included to the invertebrate collection of the Zoological Museum of the University of Helsinki.

3. Results

3.1. Recovery of species

Table 1 shows the recovery of the species as indicated by the three methods. The total number of the records on the species in all the habitats is 226 by hand-sorting method (HS), 189 records by formalin method (F) and 241 by the combination of the former two meth-

Table 1. Total numbers of recoveries of the species in the locations (n = 81) as well as those indicated by the three methods surveyed

Species		Recovery in locations	Recovery in samples			χ^2 (d.f. 1)		
			HS	F	FHS	F/HS	F FHS	HS/FHS
Eiseniella tetraedra	Σ	**14**	11	7	10	NS	NS	NS
	ad.	11	8	4	7	NS	NS	NS
	juv.	11	8	3	8	NS	NS	NS
Allolobophora rosea	Σ	**21**	18	9	17	NS	NS	NS
	ad.	19	15	6	15	3.9*	3.9*	NS
	juv.	18	16	8	17	NS	NS	NS
Allolobophora caliginosa	Σ	**29**	27	22	28	NS	NS	NS
	ad.	28	25	9	22	7.5**	5.5*	NS
	juv.	29	26	21	27	NS	NS	NS
Allolobophora chlorotica	Σ	**4**	4	2	3	NS	NS	NS
	ad.	4	4	2	3	NS	NS	NS
	juv.	2	2	0	1	NS	NS	NS
Octolasium cyaneum	Σ	**3**	3	3	3	NS	NS	NS
	ad.	3	2	0	3	NS	NS	NS
	juv.	3	3	3	3	NS	NS	NS
Octolasium lacteum	Σ	**25**	19	12	20	NS	NS	NS
	ad.	23	15	7	16	NS	NS	NS
	juv.	18	13	8	12	NS	NS	NS
Dendrobaena rubida	Σ	**23**	18	11	16	NS	NS	NS
	ad.	19	13	8	14	NS	NS	NS
	juv.	15	11	6	9	NS	NS	NS
Dendrobaena octaedra	Σ	**66**	53	45	61	NS	NS	NS
	ad.	57	45	20	46	9.6**	10.2**	NS
	juv.	57	42	37	53	NS	NS	NS
Lumbricus rubellus	Σ	**50**	39	39	43	NS	NS	NS
	ad.	38	30	22	25	NS	NS	NS
	juv.	44	32	34	39	NS	NS	NS
Lumbricus castaneus	Σ	**10**	6	8	9	NS	NS	NS
	ad.	8	6	6	6	NS	NS	NS
	juv.	9	5	8	9	NS	NS	NS
Lumbricus terrestris	Σ	**32**	28	31	31	NS	NS	NS
	ad.	24	6	21	23	8.3**	NS	10.0**
	juv.	31	28	30	30	NS	NS	NS
total	Σ	**277**	**226**	**189**	**241**	NS	6.3*	NS
	ad.	234	169	105	180	14.9***	19.7***	NS
	juv.	237	186	158	208	NS	6.8**	NS

In each location the three types of samples were taken and in addition to the total recoveries the corresponding frequencies for the adults and the juveniles are separately indicated.

Symbols: HS = hand-sorting, F = formalin application, FHS = combination of both methods. The asterisks denote statistical significance as follows. * = $0.05 > P > 0.01$. ** = $0.01 > P > 0.001$. *** = $P < 0.001$. NS = not significant. For further explanation see the text.

ods (FHS). The chi-square (χ^2) tests applied to the total frequencies showed no significant differences between F and HS or between HS and FHS, but low statistical superiority of FHS to F is indicated. In recovering any single species the methods seemed to be equal in efficiency.

If we group the individuals according to their reproductive status statistically significant differencies in the recovery of the adults of *Allolobophora rosea*, *A. caliginosa* and *Dendrobaena octaedra* were found in favour of both HS and FHS to F. On the other hand, the presence of the adults of *Lumbricus terrestris* was more often recorded by F and FHS than by HS. Considering the total recovery of the adults the superiority of HS and FHS to F was very significant, but HS and FHS seemed to be equal in this respect.

Table 2. Total recoveries of the individuals of different species by the three methods

Species		Number of individuals			χ^2 (d.f. 1)			Samples with greater number of individuals		χ^2 (d.f. 1)		Number of locations
		HS	F	FHS	HS/F	FHS/F	HS/FHS	HS/F	HS/FHS	HS/F	HS/FHS	n
Eiseniella tetraedra	Σ	33	9	31	13.7**	12.1***	NS	10/3	7/5	NS	NS	14
	ad.	17	5	18	6.5*	7.3**	NS	7/3	4/4	NS	NS	11
	juv.	16	4	13	7.2**	4.8*	NS	7/1	5/4	4.5*	NS	11
Allolobophora rosea	Σ	280	69	288	127.6***	134.4***	NS	18/1	12/8	15.2***	NS	21
	ad.	63	7	82	44.8***	63.2***	NS	15/0	9/7	15.0***	NS	18
	juv.	217	62	206	86.1***	77.4***	NS	16/1	11/7	13.2***	NS	18
Allolobophora caliginosa	Σ	338	96	362	134.9***	153.0***	NS	25/2	13/13	20.0***	NS	29
	ad.	103	16	107	63.6***	67.3***	NS	25/2	13/13	19.6***	NS	28
	juv.	235	80	255	76.3***	91.4***	NS	20/3	12/15	12.6***	NS	29
Allolobophora chlorotica	Σ	40	2	32	34.4***	26.5***	NS	3/0	3/0	NS	NS	4
	ad.	22	2	19	16.7***	13.8***	NS	3/0	2/1	NS	NS	4
	juv.	18	0	13	18.0***	13.0***	NS	2/0	1/1	NS	NS	2
Octolasium cyaneum	Σ	21	3	32	13.5***	24.0***	NS	3/0	0/2	NS	NS	3
	ad.	6	0	9	6.0*	9.0**	NS	2/0	0/3	NS	NS	3
	juv.	15	3	23	8.0**	15.4***	NS	3/0	2/1	NS	NS	3
Octolasium lacteum	Σ	73	20	79	30.2***	35.2***	NS	16/6	10/13	4.5*	NS	25
	ad.	24	8	27	8.0**	10.3**	NS	11/2	10/11	6.2*	NS	23
	juv.	49	12	52	22.4***	25.0***	NS	12/6	7/11	NS	NS	18
Dendrobaena rubida	Σ	52	28	81	7.2**	25.8***	6.3*	14/3	11/11	7.1**	NS	23
	ad.	32	18	43	3.9*	10.2***	NS	9/3	7/10	NS	NS	19
	juv.	20	10	38	NS	16.3***	5.6*	8/4	6/9	NS	NS	15
Dendrobaena octaedra	Σ	439	165	465	124.3***	142.9***	NS	41/19	21/39	8.1**	5.4*	66
	ad.	141	35	133	63.8***	57.2***	NS	38/7	23/30	21.4***	NS	57
	juv.	298	130	332	65.9***	88.3***	NS	35/16	16/34	7.1**	6.5*	57
Lumbricus rubellus	Σ	229	186	245	4.5*	8.1**	NS	26/17	21/25	NS	NS	50
	ad.	100	61	70	9.4**	NS	5.3*	24/12	23/13	NS	NS	38
	juv.	120	125	175	NS	8.3**	7.0**	17/20	12/27	NS	5.8*	43
Lumbricus castaneus	Σ	36	39	58	NS	NS	5.1*	4/6	2/8	NS	NS	10
	ad.	12	13	22	NS	NS	NS	3/4	2/5	NS	NS	8
	juv.	24	26	36	NS	NS	NS	2/4	2/6	NS	NS	9
$total_1$	Σ	1,541	617	1,673	395.6***	487.0***	5.4*	160/57	100/124	48.9***	NS	—
	ad.	520	165	530	184.0***	191.7***	NS	137/33	93/97	62.6***	NS	—
	juv.	1,021	452	1,143	219.8***	299.4***	6.9**	122/55	74/115	25.4***	8.9**	—
Lumbricus terrestris	Σ	104	209	260	35.2***	5.5*	66.9***	5/24	3/27	12.5***	19.2***	32
	ad.	9	48	55	26.7***	NS	33.1***	3/18	2/20	10.7***	14.7***	24
	juv.	95	161	205	17.0***	5.3*	40.3***	6/15	3/21	3.9*	13.5***	31
$total_2$	Σ	1,645	826	1,933	271.5***	444.2***	23.2***	165/81	103/151	28.7***	9.1**	—
	ad.	529	213	585	134.6***	173.4***	NS	140/51	95/117	41.5***	NS	—
	juv.	1,116	613	1,348	146.3***	275.5***	21.8***	128/70	77/136	17.0***	16.3***	—

For the symbols see Table 1.

Table 8. Total weights of preserved individuals of the species by the three methods

Species		Total weight (g)			F/HS	F/FHS	FHS/HS	Mean weight (g)		
		HS	F	FHS	(%)	(%)	(%)	HS	F	FHS
Eiseniella tetraedra	Σ	1.921	0.457	1.953	**23.8**	**23.4**	**101.7**	0.060	0.053	0.065
	ad.	1.535	0.352	1.465	**22.9**	**24.0**	**95.4**	0.089	0.081	0.084
	juv.	0.386	0.105	0.488	**27.2**	**21.5**	**126.4**	0.025	0.026	0.038
Allolobophora rosea	Σ	35.241	8.862	41.309	**25.1**	**21.5**	**117.2**	0.125	0.133	0.141
	ad.	17.249	2.272	23.371	**13.2**	**9.7**	**135.5**	0.310	0.325	0.290
	juv.	17.992	6.590	17.938	**36.6**	**36.7**	**99.7**	0.084	0.110	0.087
Allolobophora caliginosa	Σ	110.018	24.072	114.260	**21.9**	**21.1**	**103.9**	0.316	0.254	0.324
	ad.	64.608	10.550	63.545	**16.3**	**16.6**	**98.4**	0.637	0.682	0.647
	juv.	45.410	13.522	50.715	**29.8**	**26.7**	**111.7**	0.200	0.168	0.208
Allolobophora chlorotica	Σ	7.035	0.615	6.085	**8.7**	**10.1**	**86.5**	0.165	0.308	0.177
	ad.	5.404	0.615	4.945	**12.1**	**12.4**	**91.5**	0.216	0.308	0.254
	juv.	1.631	—	1.140	—	—	**69.9**	0.109	—	0.088
Octolasium cyaneum	Σ	13.874	2.280	22.788	**16.4**	**10.0**	**164.2**	0.915	0.837	0.742
	ad.	8.442	—	12.313	—	—	**145.9**	1.363	—	1.445
	juv.	5.432	2.280	10.475	**42.0**	**21.8**	**192.8**	0.659	0.837	0.526
Octolasium lacteum	Σ	19.955	6.289	24.966	**31.5**	**25.2**	**125.1**	0.216	0.314	0.296
	ad.	15.221	4.622	17.246	**30.4**	**26.8**	**113.3**	0.706	0.578	0.615
	juv.	4.734	1.667	7.720	**35.2**	**21.6**	**163.1**	0.102	0.139	0.147
Dendrobaena rubida	Σ	6.018	4.253	8.963	**70.7**	**47.5**	**148.9**	0.122	0.152	0.113
	ad.	4.323	2.991	6.575	**69.2**	**45.5**	**152.1**	0.136	0.195	0.172
	juv.	1.695	1.262	2.388	**74.5**	**52.8**	**140.9**	0.097	0.075	0.051
Dendrobaena octaedra	Σ	28.050	10.284	29.537	**36.7**	**34.8**	**105.3**	0.065	0.062	0.065
	ad.	14.622	3.906	14.524	**26.7**	**26.9**	**99.3**	0.110	0.114	0.120
	juv.	13.428	6.378	15.013	**47.5**	**42.5**	**111.8**	0.045	0.049	0.046
Lumbricus rubellus	Σ	92.706	74.186	89.135	**80.0**	**83.2**	**96.1**	0.438	0.403	0.376
	ad.	68.255	46.006	50.025	**67.4**	**92.0**	**73.3**	0.814	0.763	0.755
	juv.	24.451	28.180	39.110	**115.3**	**72.1**	**160.0**	0.191	0.227	0.228
Lumbricus castaneus	Σ	2.479	2.900	4.031	**117.0**	**71.9**	**162.6**	0.073	0.074	0.072
	ad.	1.226	1.586	2.326	**129.4**	**68.2**	**189.7**	0.102	0.122	0.115
	juv.	1.253	1.314	1.705	**104.9**	**77.1**	**136.1**	0.056	0.051	0.050
$total_1$	Σ	317.297	134.198	343.027	**42.3**	**39.1**	**108.1**	0.198	0.219	0.205
	ad.	200.885	72.900	196.335	**36.3**	**37.1**	**97.7**	0.388	0.457	0.384
	juv.	116.412	61.298	146.692	**52.7**	**41.8**	**126.0**	0.115	0.134	0.130
Lumbricus terrestris	Σ	81.900	303.535	354.164	**370.6**	**85.7**	**432.4**	0.717	1.481	1.392
	ad.	31.547	194.944	222.567	**617.9**	**87.6**	**705.5**	2.794	4.120	4.118
	juv.	50.353	108.591	131.597	**215.7**	**82.5**	**261.4**	0.500	0.680	0.663
$total_2$	Σ	399.197	437.733	697.191	**109.7**	**62.8**	**174.6**	0.227	0.541	0.378
	ad.	232.432	267.844	418.902	**115.2**	**63.9**	**180.2**	0.431	1.300	0.789
	juv.	166.765	169.889	278.289	**101.9**	**61.0**	**166.9**	0.143	0.277	0.215

For the symbols see Table 1.

Among the juveniles no significant differences between the three methods were found in any single species, but the totals of the juveniles indicated the superiority of FHS to F.

As far as the mere presence of the species in the samples is concerned HS and FHS can be regarded to be equal in efficiency suggesting that FHS have had no drawbacks as compared either to HS or F.

3.2. Recovery of individuals

The total numbers of the individuals by the three methods very strongly suggest the superiority of HS and FHS to F (Table 2, $total_2$). This is also true within the species excluding *Lumbricus castaneus* in which F was equal in efficiency to HS and to FHS and *L. terrestris* in which both F and FHS were more efficient than HS. No statistical differences between HS and FHS were found when the total numbers of the individuals of *Eiseniella*, *Allolobophora* and *Octolasium* species were considered. This is also true in *Dendrobaena octaedra* and *Lumbricus rubellus*, but in *Dendrobaena rubida* and *Lumbricus castaneus* FHS yielded somewhat higher numbers of individuals than HS. Excluding the numbers of *Lumbricus terrestris* individuals FHS can be regarded only slightly superior to HS (Table 2, $total_1$) but the difference becomes statistically more significant when the numbers of *L. terrestris* are added ($total_2$).

Moreover, the frequencies of the samples with greater number of individuals between the methods compared in pairs warrant us to conclude that in all the species except in *L. terrestris* HS is superior or at least equal in efficiency to F, and in all the species FHS is superior or equal to HS. The difference in the totals of the last two methods is mostly due to the low efficiency of HS to recover individuals of *L. terrestris*.

Considering the total numbers of the adults in *Eiseniella*, *Allolobophora*, *Octolasium* and *Dendrobaena* species F is inferior both to HS and FHS, but HS and FHS seem to be equal in this respect. In *Lumbricus castaneus* the numbers of the adults by the three methods are rather low indicating no significant differences between the methods. HS yielded more adults of *Lumbricus rubellus* than either FHS or F, but the frequencies of the samples with greater number of individuals indicate no statistical differences between the methods. In *L. terrestris* the superiority of F and FHS to HS was doubtless.

The totals of the adults by the methods show that FHS recovered more individuals than the other two methods though the difference between HS and FHS indicate no statistical significance. This also holds for the frequencies of the samples with greater number of adults.

The numbers of the juveniles of *Eiseniella*, *Allolobophora*, *Octolasium* and *Dendrobaena* species by F are much lower than those by HS or FHS, but the last two methods seemed to be rather equal in extracting the juveniles of these species. In *L. rubellus* and in *L. terrestris* FHS recovered more juveniles than either F or HS, in *L. castaneus* the methods were equal but the numbers of the juveniles were rather low.

The number of the juveniles ($total_1$, Table 2) by FHS was higher than that by HS and the difference becomes statistically even more significant when the number of the juvenile *L. terrestris* individuals is added ($total_2$). This also applies to the frequencies of the samples with greater number of juveniles.

We may conclude that of the three methods surveyed FHS has extracted the highest number of individuals and that in all the species the statistical tests applied indicate the superiority or at least the equality of FHS to F and HS.

3.3. Weight of individuals

The estimates of the total biomass of the species by the three methods were based on weighings of groups of preserved individuals. The superiority of HS to F was demonstrated in all the species except in *L. castaneus* and *L. terrestris* (Table 3). It is worth noting that

Table 4. Mean number of the species recorded in the five types of habitats by the three methods

Habitats	Number of loc. sampled	Mean number of species					
		HS		F		FHS	
		$\bar{x} \pm$ S.E.	range	$\bar{x} \pm$ S.E.	range	$\bar{x} \pm$ S.E.	range
Alluvial sea shores	14	1.86 ± 0.33	0–4	0.93 ± 0.32	0–4	1.71 ± 0.34	0–4
Shore alder thickets	13	2.62 ± 0.33	1–5	2.77 ± 0.43	1–6	3.23 ± 0.44	1–6
Rich forests	24	3.50 ± 0.25	1–6	3.63 ± 0.29	1–6	4.08 ± 0.32	2–7
Meadows	13	4.46 ± 0.35	3–7	3.23 ± 0.40	1–6	4.15 ± 0.42	2–6
Coniferous forests	17	1.35 ± 0.21	0–3	0.65 ± 0.15	0–2	1.35 ± 0.27	0–5
total	81	2.78 ± 0.18	0–7	2.33 ± 0.20	0–6	2.98 ± 0.20	0–7

In each location one sample unit equals 1/8 m². For the symbols see Table 1.

Table 5. Relative efficiencies of the three methods in recovering Lumbricidae individuals in five types of habitats in S Finland

Habitats	Number of loc. sampled	Number of individuals			χ^2 (d.f. 1)			Number of samples with greater number of individuals		χ^2 (d.f. 1)	
	n	HS	F	FHS	HS/F	FHS/F	FHS/HS	HS/F	HS/FHS	HS/F	HS/FHS
Alluvial sea shores	14	86	22	83	37.9***	35.4***	NS	9/3	8/4	NS	NS
Shore alder thickets	13	221	137	278	19.7***	47.9***	6.5*	10/2	3/9	5.3*	NS
Rich forests	24	577	398	758	32.9***	112.1***	24.5***	18/4	4/20	8.9**	10.7**
Meadows	13	566	238	651	133.8***	191.9***	5.9*	13/0	2/11	13.0***	6.2*
Coniferous forests	17	195	31	163	119.0***	89.8***	NS	14/1	7/7	11.3***	NS
total	81	1,645	826	1,933	271.5***	444.2***	23.2***	64/10	24/51	39.4***	9.7**

For the symbols see Table 1 and for the relative efficiency of the methods on each species in different habitats see Figs. 1 and 2.

the biomass of the latter species by HS was about three times lower than that by F or FHS, and that is why the estimate of the total biomass by HS differs much from those by F or FHS.

In all the species except in *A. chlorotica* and *L. rubellus* the total weights of the individuals by FHS exceeded those by HS. In these two species the difference in favour of HS to FHS was only 3.9 % and 13.5 %, respectively.

Excluding *L. castaneus* and *L. terrestris* the biomass of the adults by F were lower than those by HS. Although in some of the species HS yielded higher estimates of the biomass of the adults than FHS the former method is inappropriate to estimate the biomass of the adults of *L. terrestris*. This restricts the adoption of HS to habitats where *L. terrestris* does not occur.

In *L. rubellus* the difference in favour of HS to FHS was at its greatest viz. 26.7 %. To recover the adults of the other species HS and FHS can be regarded to be equal as far as the biomass is considered.

In the juveniles the estimates by HS were somewhat higher than those by F in all the species but not in those belonging to the genus *Lumbricus*, and in all the species excluding *A. chlorotica* the biomasses by FHS exceeded those by HS.

In most species the mean weights of the adults or the juveniles do not indicate any great differences between the methods suggesting that the efficiency of the methods adopted, including the accuracy of the operator, had no selective impact upon the recovery of the individuals of different size. In *L. terrestris* both the adults and the juveniles found by F and FHS were on an average heavier than those by HS. Evidently, this is due to the efficiency of formalin application to expel big individuals of this species from the depth of the soil laborious to reach by digging.

3.4. Efficiency of the methods in different habitats

3.4.1. Recovery of species

Table 4 shows the recovery of the species by the methods in the five types of habitats. In each habitat the differences in the mean numbers of the species indicate no statistica differences between the methods. In alluvial sea shores and in coniferous forests F recovered only about one half of the numbers of the species by HS or FHS, but in shore alder thickets, rich forests and meadows the corresponding rate of recovery by F was higher. This is mostly due to the differences in the species composing the Lumbricidae fauna of the habitats in responding to formalin solution and not to e.g. the differences in the structure of the soils.

3.4.2. Recovery of individuals

Table 5 shows the numbers of the individuals extracted by the three methods in the five types of habitats. In each habitat there was a wide range of the numbers of the individuals present in different locations and that is why instead of counting the mean densities I prefer to indicate the total numbers of the individuals and the numbers of the samples with greater number of individuals by the methods compared in pairs.

In alluvial sea shores and in coniferous forests the efficiency of F was about 25 % or less compared to that of HS or FHS. In these habitats HS and FHS were rather equal in efficiency, and this is also true if the numbers of the samples with greater number of individuals are considered.

In habitats rich with organic soil FHS seems to be more efficient than HS. In rich forests in 20 of the 24 locations sampled FHS yielded more individuals than HS, in meadows the corresponding numbers were 11 out of 13 and in shore alder thickets 9 out of 13. In one location of the latter habitat both FHS and HS recovered the same number of individuals.

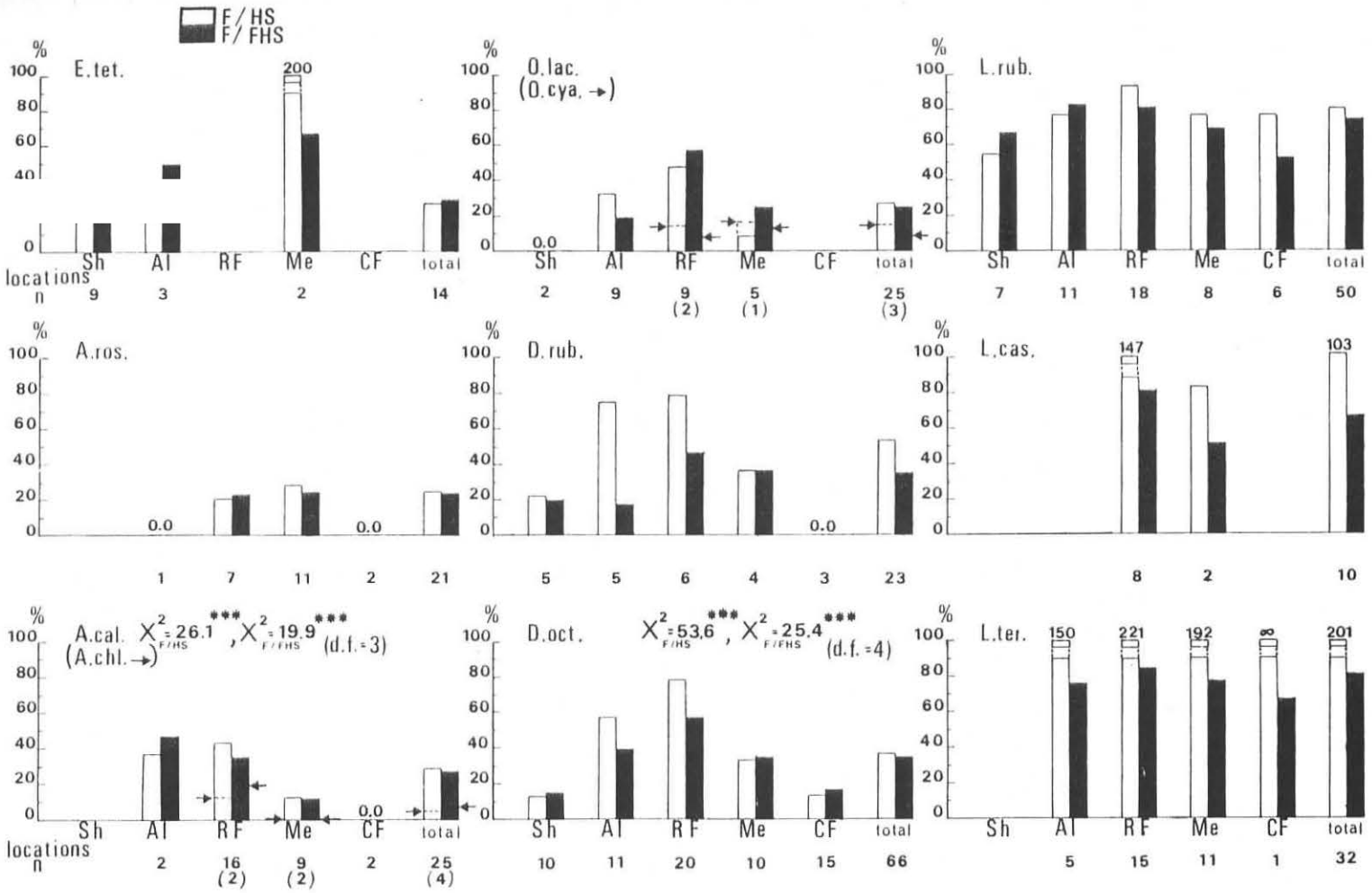

Fig. 1. Efficiency of F compared to that of HS and FHS in extracting individuals of Lumbricidae species in five types of habitats in S Finland. Symbols: Sh = alluvial sea shores, Al = shore alder thickets, RF = rich forests, Me = meadows and CF = coniferous forests. For further information tee the text.

Fig. 1 indicates the relative efficiency of F to expel the individuals of each species in different habitats. The chi-square tests applied to the numbers of the individuals show that in *A. caliginosa* and *D. octaedra* the relative efficiency of F has not been equal in all the habitats. F has extracted individuals of *A. caliginosa* with low efficiency on an average and e.g. in coniferous forests, where the species was recorded twice, no individuals were recovered by F. On the other hand, in shore alder thickets the efficiency of F to extract *A. caliginosa* was highest viz. about 40 % compared to that of FHS or HS.

D. octaedra is very constant and often rather abundant in coniferous forests in S. Finland, but the efficiency of F is low in this habitat likewise it is in shore alder thickets. On the other hand, in habitats rich with organic soil the relative efficiency of F in extracting *D. octaedra* is somewhat higher.

In the latter two species the differences found maybe due to physical and/or chemical properties of the soils or these in turn may have had an unequal effect upon e.g. the activity pattern of the individuals in different habitats with the result that the individuals of the same species respond unequally to formalin solution applied to the soil.

In all the other species of Fig. 1 the material is homogeneous indicating that no great changes in the relative rate of recovery of the individuals by the methods has taken place when the habitats were sampled.

Considering the relative efficiencies of the methods within each habitat over all the species in Fig. 1 statistically significant differences between F/HS and F/FHS were found in shore alder thickets ($\chi^2_{F/HS} = 23.1^{**}$, d.f. 7, $\chi^2_{F/FHS} = 23.5^{**}$, d.f. 7), meadows ($\chi^2_{F/HS} = 130.7^{***}$, d.f. 10, $\chi^2_{F/FHS} = 72.3^{***}$, d.f. 10), rich forests ($\chi^2_{F/HS} = 112.2^{***}$, d.f. 9, $\chi^2_{F/FHS} = 54.8^{***}$, d.f. 9) and coniferous forests ($\chi^2_{F/HS} = 43.5^{***}$, d.f. 5, $\chi^2_{F/FHS} = 14.1^{**}$, d.f. 5). In most of the habitats the recovery rate of the individuals by F was much lower in *Eiseniella*, *Allolobophora*, *Octolasium* and *Dendrobaena* species than the corresponding rate among the individuals belonging to the genus *Lumbricus*. This strongly suggests the high selective efficiency of formalin solution to expel the individuals of different species.

Fig. 2 shows the efficiency of FHS compared to that of HS in each species recorded in different habitats. Statistically significant differences indicating the heterogeneity of the material were found in *O. lacteum* and *D. octaedra*. In the former species the efficiency of FHS was lowest in habitats rich with organic soil but in the latter one it was highest there. In neither of them the heterogeneity was high ($0.05 > P > 0.01$) and I prefer not to draw any further conclusions.

Considering each habitat over all the species in Fig. 2 statistically significant differences in the relative efficiencies of the two methods compared were found in shore alder thickets ($\chi^2_{FHS/HS} = 24.4^{***}$, d.f. 7), meadows ($\chi^2_{FHS/HS} = 26.3^{***}$, d.f. 10), rich forests ($\chi^2_{FHS/HS} = 32.2^{***}$, d.f. 9) and coniferous forests ($\chi^2_{FHS/HS} = 12.6^{*}$, d.f. 5). The differences are for a great deal due to the higher efficiency of FHS to extract individuals of *L. terrestris*.

Figs. 1 and 2 indicate that the three methods have recovered the individuals of the species with unequal efficiency in different habitats, but within most species the relative efficiency of the methods was equal in all the habitats. This seems to suggest that in each habitat the total number of the individuals extracted by any of the three methods was largely accounted for by the numbers of the individuals present in the habitat as well as the species which the individuals belonged to and it was not much influenced by e.g. different properties of the soils.

Bouché (1969) pointed out that the efficiency of formalin application to expel the individuals of the same species is not equal in different spells of the year. It seems that most of the individuals not expelled by formalin solution can be recovered by hand-sorting in habitats with mineral soil not lying very deep, but in habitats with thick layer of organic soil and presence of deep-burrowing species, especially *L. terrestris*, changes in the efficiency of formalin solution to expel deep-burrowing species may also lead to underestimation of the standing crops of the Lumbricidae populations.

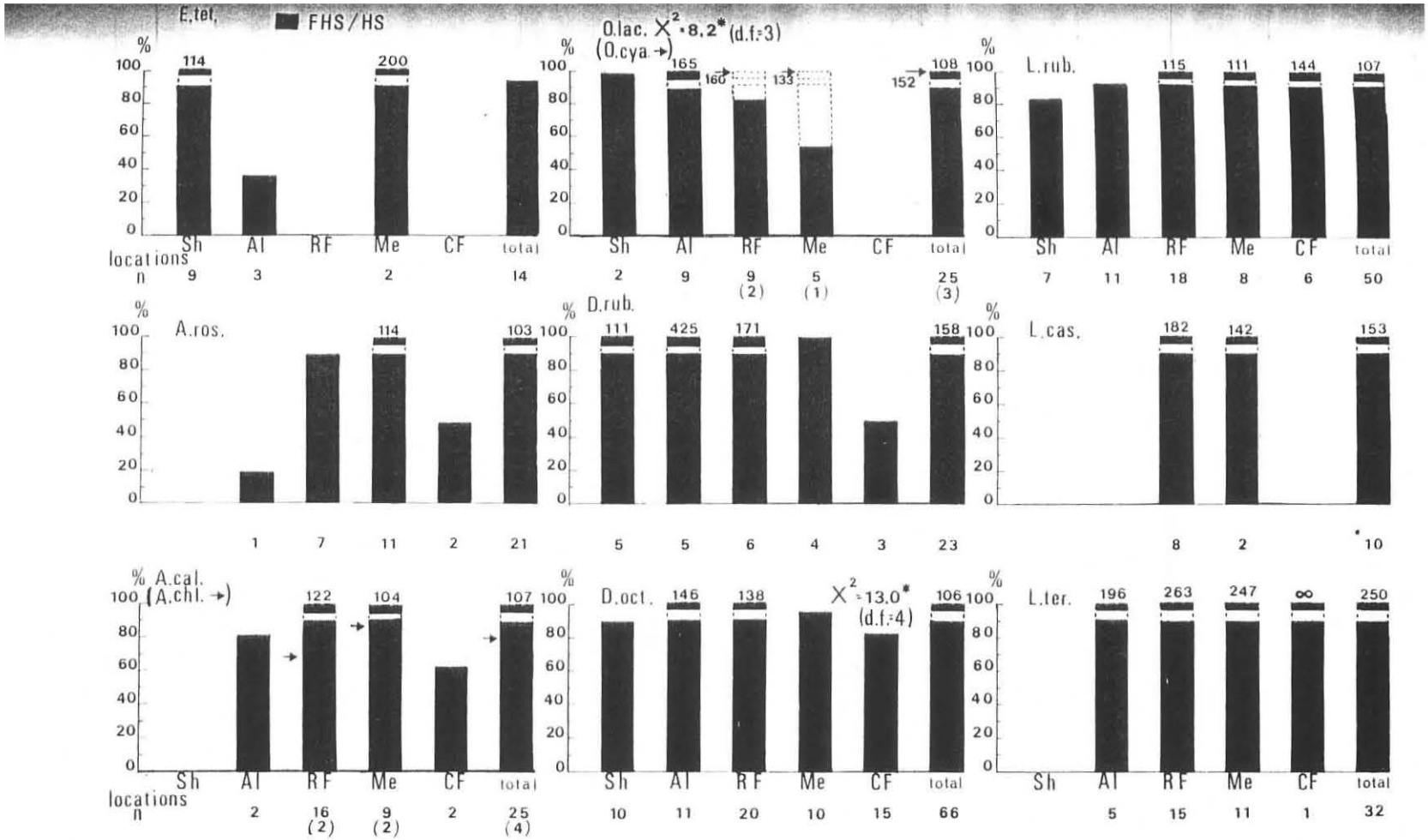

Fig. 2. Efficiency of FHS compared to that of HS in recovering individuals of Lumbricidae species in five types of habitats in S Finland. For the symbols see Fig. 1.

3.4.3. *Weight of individuals*

Considering the total biomasses of the earthworms by the methods in different habitats (Table 6) there is no doubt about the superiority of FHS to F or HS. Due to their higher efficiency to recover big individuals of *L. terrestris* both FHS and F have yielded higher estimates of the total biomass than HS in habitats rich in organic soil.

In coniferous forests HS and FHS were almost equal in efficiency but on alluvial sea shores the difference in favour of FHS is due to somewhat higher number of the adults of *L. rubellus* recovered by FHS.

Table 6. Total biomass of the Lumbricidae sampled in five types of habitats by the three methods surveyed in S. Finland

Habitats	Number of loc. sampled	Total biomass			Relative biomass		
		HS	F	FHS	F/HS	F/FHS	FHS/HS
Alluvial sea shores	14	11.259	7.142	14.733	*63.4*	*48.5*	*130.9*
Shore alder thickets	13	76.625	57.522	95.559	*75.1*	*60.2*	*124.8*
Rich forests	24	169.553	227.831	327.654	*134.4*	*69.5*	*193.2*
Meadows	13	113.040	138.831	230.601	*122.8*	*60.2*	*204.0*
Coniferous forests	17	28.720	6.407	28.644	*22.3*	*22.4*	*99.7*
total	81	399.197	437.733	697.191	*109.7*	*62.8*	*174.6*

For the symbols see Table 1.

4. Discussion

No doubt, each of the methods surveyed has its drawbacks. Hand-sorting is often used for assessing the efficiency of other methods and, undoubtedly, the accuracy of the operator is utterly important. Re-sorting or using different kinds of mechanical apparatus to re-check the soil material more individuals can be found, but the present material strongly suggests that samples taken from habitats with *L. terrestris* by digging up the soil material yield underestimates both of the total biomass and in the total numbers of individuals. Accordingly, it is suggested that in habitats rich in organic soil F and HS are replaced by FHS.

One may think whether the superiority of FHS to HS is due to the formalin application which may also have expelled some individuals lying outside the sample plot but having their burrows opening to the surface of the soil inside the sample frame. On the other hand, formalin solution not only runs along the burrows but also diffuses right through the soil and that in turn may have expelled some individuals inside the sample plot to come to the surface outside it. In all the adults of the species except in *L. terrestris* HS and FHS were rather equal in efficiency and this is also true if the totals of the adults in Table 2 are considered. This suggests that in these species no migration of the adults out or into the sample plot has taken place. The numbers of the adults of *L. terrestris* by F and FHS are about 5—7 times higher than that by HS and much the same rate of difference is also indicated by Raw (1959, Table 1), but, among the juveniles not moving so fast the difference is much lower. Moreover, in the field one has to pick up very quickly the big individuals of *L. terrestris* expelled by formalin solution, and, if missed, they quickly disappear into the soil and are not recovered by handsorting the soil material. No doubt, the big individuals of *L. terrestris* are capable of moving quickly in the soil.

Supposing that HS in fact has yielded adequate numbers of *L. terrestris* individuals present in the sample plot we have to accept that all the other individuals expelled by F or FHS have come outside the plot. Accordingly, the numbers of the individuals recovered by F or FHS in one $^1/_8$ m^2 unit would then roughly correspond to the numbers of the individuals revealed by HS in one square metre. This cannot be true, but further studies on the efficiency of chemical methods to extract quickly moving species with long burrows should be made.

FHS also extracted more or at least equal numbers of juveniles than HS. Many of the juveniles stay between the roots of grasses and herbs and they are not always easy to record there. Formalin solution expels many of them to the surface of the soil or at least it makes them stretch out and possibly that is why they are more often recorded by FHS than by HS.

In FHS method the time spent after formalin application may not be too long, possibly the 5 minute-period adopted is enough, since the longer it is the higher the number of the individuals being able to avoid the recovery by moving out of the sample plot inside the soil.

In habitats with thin layer of organic soil and often accompanied with low pH the adoption of HS instead of F or FHS is recommended. At least in sea shore habitats with dense mat of roots of grasses and herbs and with heavy clay formalin application wets the soil and makes it difficult to handsort.

The study material was sampled in 1977 and 1979. To indicate possible differences between the two years chi-square tests were applied to the total numbers of the individuals by the methods within each habitat. In rich forests low statistical heterogeneity of the material between the two years was indicated when F was compared to HS ($\chi^2_{1977/79} = 4.3^*$, d.f. 1) or to FHS ($\chi^2_{1977/79} = 4.6^*$, d.f. 1). In all the other habitats the material was homogeneous in this respect.

Comparing the efficiency of FHS to that of HS in different habitats no significant differences between the two years were found. This seems to suggest that in rich forests the differences between the two years are mostly due to differences in the efficiency of formalin solution in expelling the individuals present there.

In the present study the soil of the samples was not re-sorted, thus the numbers of the individuals recovered should be considered as the minimum numbers of individuals present in the samples. By comparing these estimates to the corresponding values in the literature one may get some rough idea about the efficiency of the methods applied in different habitats.

Accordingly, in the present study the mean density (ind./m²) with corresponding standard error (S.E.) in coniferous forests was 91.8 ± 21.9 by HS and 76.7 ± 14.6 by FHS. Huhta et al. (1967) recorded 28.9 ind./m² and 57 ind./m² in MT and OMT forests in S. Finland, respectively. The wet funnel type of extractor adopted by Huhta & Koskenniemi (1975) recovered 117 ind./m² from raw humus soils in MT forests and 89 ind./m² in OMT forests in S Finland.

In rich forests the range of the numbers of the Lumbricidae individuals in one square metre is very wide, e.g. Karppinen (1958) extracted 52—346 ind./m², the mean being 214 ind./m² from OMaT forests in S. Finland. In the present study the corresponding figures in rich forests are 24—816 ind./m² and $\bar{x}$ ± S.E. = 252.7 ± 37.0 ind./m² by FHS.

In alluvial sea shores, shore alder thickets and meadows the mean densities of individuals ($\bar{x}$ ± S.E.) are 49.1 ± 12.4, 136.0 ± 28.3, 348.3 ± 59.3 by HS and 47.4 ± 13.7, 171.1 ± 38.1, 400.6 ± 65.2 by FHS, respectively. Unfortunately, no Finnish data for comparison are available.

The material examined seems to warrant us to draw the following conclusions: handsorting and formalin application can be combined into one sample procedure without any drawbacks in the efficiency when compared either to F or HS. In Finland in habitats rich with organic soil and presence of deep-burrowing species the adoption of FHS instead of F or HS is recommended, but in habitats with rather thin layer of organic soil and absence of deep-burrowing species formalin application prior to hand-sorting is not necessary.

5. Summary

The present study was aimed at assessing the relative efficiency of three sample methods viz. hand-sorting (HS), formalin application (F) and combination of the above methods (FHS) in extracting Lumbricidae from different types of habitats in S. Finland.

Considering the total numbers of the records indicating the presence of the species in the locations sampled HS and FHS were equal in efficiency but low superiority of FHS to F was found. Comparing the corresponding numbers of recoveries among the adults HS and FHS were superior to F but no significant difference between the two methods mentioned first was found. In the total recoveries of the juveniles FHS was superior to F but no other statistical differences between the methods were recorded.

In recording the presence of any single species in the samples the methods were equal in efficiency, but if the adults or the juveniles within each species are separately considered differencies were indicated among the adults of *A. rosea*, *A. caliginosa* and *D. octaedra* in favour of FHS and HS to F. The presence of the adults of *L. terrestris* was more often revealed by F and FHS than by HS.

Considering the total numbers of the individuals, or those of the adults and the juveniles separately, highly significant statistical differences between the methods were found in most species. In *E. tetraedra*, *A. rosea*, *A. caliginosa*, *A. chlorotica*, *O. cyaneum*, *O. lacteum*, *D. rubida* and *D. octaedra* F was inferior to HS and FHS, but in these species the last two methods were rather equal in efficiency. In *Lumbricus* species the totals of the individuals by the methods indicated the equality or the superiority of FHS both to HS and F. Especially, in *L. terrestris* HS proved to be very inefficient to extract both the adults and the juveniles when compared to FHS.

In all the species except in *A. clorotica* and *L. rubellus* the total weights of the preserved individuals by FHS were higher than those by HS or F. In these two species the differences in favour of HS to FHS were not great. In *L. terrestris* the total biomass by HS was 3.7 and 4.3 times lower than that by F and FHS, respectively. No doubt in habitats with *L. terrestris* HS should be replaced by FHS if the biomass of the Lumbricidae populations are estimated.

The methods surveyed recovered individuals of Lumbricidae species with unequal efficiency in different habitats, but within most species no statistical differences in the relative efficiency of the methods in different habitats were found. One has to conclude that in the habitats sampled the total number of the individuals extracted by each method was mostly due to the numbers of the individuals present there as well as to the species which the individuals belonged to i.e. physical and chemical properties of the soils seem to have no great impact upon the relative efficiency of the methods in different habitats in S Finland, at least.

The superiority of FHS to HS in habitats rich in organic soil was mostly due to the formalin application expelling quickly moving, deep-burrowing individuals of *L. terrestris*.

In habitats with thin layer of organic soil e.g. in alluvial sea shores and coniferous forests HS and FHS were equal in efficiency and both were superior to F.

In habitats rich in organic soil e.g. in meadows, rich forests and shore alder thickets and presence of deep-burrowing species FHS was superior both to HS and F. Accordingly, the adoption of FHS instead of HS or F in these habitats is recommended.

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7. References

AXELSSON, D., U. GÄRDEFORS, T. LOHM, O. PERSSON & O. TENOW, 1971. Reliability of estimating standing crop of earthworms by hand sorting. Pedobiologia **11**, 338—340.

BOUCHÉ, M. B., 1969. Comparaison critique de méthodes d'évaluation des populations de Lombricidés. Pedobiologia **9**, 26—34.

HAEGGSTRÖM, C.-A., & J. TERHIVUO, 1979. The Lumbricidae (Oligochaeta) in the archipelago of Aland, SW Finland. Mem. Soc. Fauna Flora Fennica **55**, 17—31.

HUHTA, V., M. NURMINEN & A. VALPAS, 1967. Effect of silvicultural practices upon arthropod, annelid and nematode populations in coniferous forest soil. Ann. Zool. Fennici **4**, 87—143.

— & A. KOSKENNIEMI, 1975. Numbers, biomass and community respiration of soil invertebrates in spruce forests at two latitudes in Finland. Ann. Zool. Fennici **12**, 164—182.

KARPPINEN, E., 1958. Lierolajeistamme ja niiden esiintymisestä metsämaassa. Luonnon Tutkija **62**, 137—144.

NELSON, J. M., & J. E. SATCHELL, 1962. The extraction of Lumbricidae from soil with special reference to the hand-sorting method. In: MURPHY, P. W. (ed.): Progress in Soil Zoology, 294—299, London.

RAW, F., 1959. Estimating earthworm populations by using formalin. Nature (London) **184**, 1661 to 1662.

ZICSI, A., 1962. Determination of number and size of sampling unit for estimating lumbricid populations of arable soils. In: MURPHY, P. W. (ed.): Progress in Soil Zoology, 68—71, London.

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